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Immunotherapy of Prostate Cancer With Genetically Enhanced Tumor-Specific T Cell Precursors

PRINCIPAL INVESTIGATOR:
Marcel van den Brink, MD, PhD

CONTRACTING ORGANIZATION:
Sloan-Kettering Institute for Cancer Research
New York, NY 10065

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14. ABSTRACT
Adaptive immunity can contribute significantly to tumor immunosurveillance and anti-tumor activity of cancer treatment regimens. We have reported previously that in vitro generated T cell precursors can be adoptively transferred across MHC barriers to allogeneic hosts and demonstrated that genetically engineered tumor-specific T cell precursors mediate enhanced anti-tumor responses. In this project we have proposed to generate genetically engineered prostate cancer-specific T cell precursors and test their efficacy for tumor immunotherapy in mouse models of prostate cancer. We now report that we have established non-myeloablative regimens to support engraftment of T cell precursors and established a prostate cancer model to test our adoptive therapy strategy. Non-myeloablative regimens were developed using a combination of radiation limited to the thymic region and cytoxan chemotherapy. The prostate cancer model (both localized and disseminated) was established using inoculation of RM1 cell line expressing human prostate specific membrane antigen (PSMA). We also report that we have optimized the transduction of T cell precursors with chimeric antigen receptor (CAR) targeting PSMA- pZ1.

15. SUBJECT TERMS
T cell precursor, OP9-DL1, Adoptive immunotherapy, transduction, chimeric antigen receptor

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1. INTRODUCTION

Broader use of individual cell therapies are restricted by complex and expensive processes to manufacture a cell product that is limited to single patient use. Recent evidence suggests that T cell precursors can potentially be used for cell-based immunotherapy across MHC barriers(*1, 2*). For an effective antitumor therapy, T cell precursors could be genetically engineered to target tumor-associated antigens without depending on MHC-based presentation of antigen. The *goal of this project* is to extend the possibilities of curing malignant states using the immune system. *The overall objective of this application* is to develop an effective 'off the shelf' T cell precursor-based therapeutic strategy targeting prostate cancer. Based on studies in our and other laboratories we have *hypothesized* that genetically engineered T cell precursors expressing chimeric antigen receptors targeting prostate specific membrane antigen (PSMA) (*3*) can be adoptively transferred to enhance tumor killing in prostate cancer-bearing recipients.

2. BODY

2.1 *Engraftment of T cell precursors in the thymus:* Ex vivo generated T cell precursors require thymic niches in order to engraft into the thymus and mature into T cells. In the preclinical studies conducted hitherto, these niches were created by myeloblative regimens followed by allo/syngeneic hematopoietic transplantation. However, solid tumors like prostate cancer commonly do not require treatment with allogeneic hematopoietic cell transplantation. In order to make the 'off the shelf' immunotherapy more clinically relevant, we would develop non-myeloablative conditioning regimens precluding the need of stem cell transplants. In our initial experiments significant engraftment was achieved only with high dose total body irradiation (TBI) (Figure 1). We therefore standardized targeted irradiation of the thymic region and achieved up to 15% T cell precursor engraftment in the thymus (Fig 1C). We then used a conditioning regimen based on Cytoxan and low dose irradiation through which up to 20% engraftment of T cell precursors was achieved (Fig 1D).

2.2. *Development of mouse models of human PSMA expressing tumors:*
The laboratory of Dr. Sadelain has engineered the mouse prostate cancer cell line RM1 to express human PSMA (RM1-PSMA). Using this cell line for tumor challenges in sublethally irradiated C57BL/6 mice, we have established a prostate cancer model that results in tumor death 20 to 30 days after challenge (Figure 2A). RM1-PSMA cells expressing luciferase are also available. These cells express luciferase that catalyzes oxidation of luciferin, which can cause bioluminescence of the areas inhabited by the cells. Visualization and quantification of progressive tumor can be done over time using in vivo imaging systems (IVIS)(Figure 2B).

2.3 Genetic engineering of hPSMA-specific T cell precursors.
T-cell precursors can be engineered to stably express a chimeric antigen receptor (CAR) using viral vectors (Figure 3). The Pz 1 expression lentiviral vector was designed in our collaborating laboratory led by Dr. Michel Sadelain. T cell precursors were developed *in vitro* from C57BL/6 BM derived HSCs using the OP9-DL1 culture system, as described above. Viral vectors were produced by tripartite transfection of 293 T cells with transfer genes (Pz 1), pCMV ΔR8.92, and pUCMD.G[53] using 293 Transit (Mirius bio). Vector supernatants were concentrated by ultra-centrifugation and 0.75-1.5 x 10^8 total TU used to transduce 5 x 10^5 T cell precursors (co-culture day 4–6) over 2 days in tissue culture plates coated with retronectin. Transduced cells were then expanded for an additional 14-21 days by OP9-DL1 co-culture. Transduction efficiencies of the T cell precursors were determined by flow cytometric analysis and were routinely in the range of 60% (Figure 3B).

3. KEY RESEARCH ACCOMPLISHMENTS

1. Development of non-myeloablative conditioning regimens to support T cell precursor-based therapy using irradiation to the thymic region and cyclophosphamide.
2. Development of localized and disseminated prostate cancer model expressing human PSMA in a non-myeloablative conditioning setting using RM1-PSMA model.
3. Development of prostate cancer model where tumor burden can be visualized and quantified independent of survival using firefly Luciferase expressing RM1-PSMA.
4. Optimization of transduction of T cell precursor with Pz1 expression vector.

4. REPORTABLE OUTCOMES

-None-

5. CONCLUSION

The beneficial effects of T cell precursors that can be obtained from donors independent of their MHC-match, lead to the possibility of universal donors and "off-the-shelf" use. Engineering T cell precursors to express tumor antigen-specific chimeric antigen receptor can potentially enhance anti-tumor activity.

In the present study, we extend our previous findings to prostate cancer, based on treatment regimens independent of myeloablation, which commonly lead to significant morbidity and mortality. This strategy would for the first time allow the use of allogeneic prostate cancer-specific T cells that can be generated and stored for immediate "off- the-shelf" use. These studies are important not only in the context of immunotherapy prostate cancer, which we specifically address, but also other solid tumors, which can be similarly targeted. In the event of successful outcome, this study will be taken up in clinical trials. In the event of unexpected outcomes, their reasons would be actively pursued and tackled. This could leave an impact in the future strategies of cell-based immunotherapy.

Implications and outlook: Within the planned schedule, we have worked towards optimizing our tumor model and non-myeloablative regimens for adoptive transfer. These models will be further optimized to adapt adaptive transfers with allogeneic T cell precursors. We have also optimized transduction of T cell precursors with CAR against human PSMA. We will use these tools to test adoptive transfer of "off the shelf" T cell precursors expressing CAR targeting prostate cancer associated PSMA. Subsequently, mechanisms facilitating and hindering anti-tumor effects of this therapy will be drawn out, as outlined in the statement of work.

6. REFERENCES

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2. J. L. Zakrzewski *et al.*, *Nat Biotechnol* **26**, 453 (Apr, 2008).
3. T. P. Gade *et al.*, *Cancer Res* **65**, 9080 (Oct 1, 2005).
4. J. L. Zakrzewski *et al.*, *Nat Med* **12**, 1039 (Sep, 2006).

7. APPENDICES

-None-

8. SUPPORTING DATA

Figure 1

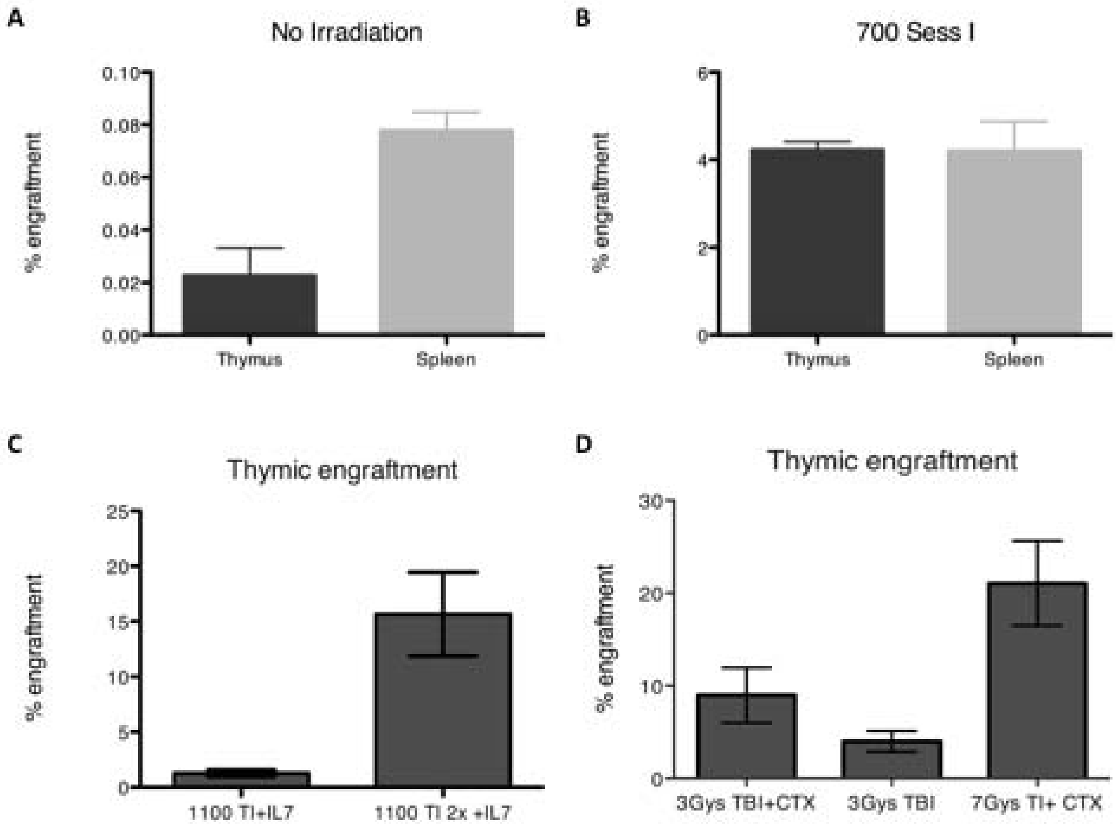

Figure 1: Engraftment of OP9-DL1 derived T cell precursors in the thymus. T cell precursors from B6 mice expressing Ly5.1 congenic marker (CD45.1) was transplanted into B6 (CD45.2) mice. The cells were transplanted with different conditioning strategies and the thymocytes and splenocytes of the recipients were analyzed 14 days following transplantation by flow cytometric anaylsis of CD45.1 (donor) and CD45.2 (recipient) cells. (n =3-5/gp) (A) No irradiation (B) 700 cGys irradiation: single dose 6 hrs before transplant (C) 1100 cGys irradiation of the thymic region either 3 days or 3 days and 6 hours before transplant. (D) 20mg/kg Cytoxan with 3Gys TBI or 7 Gys irradiation to the thymic region as indicated.

Figure 2

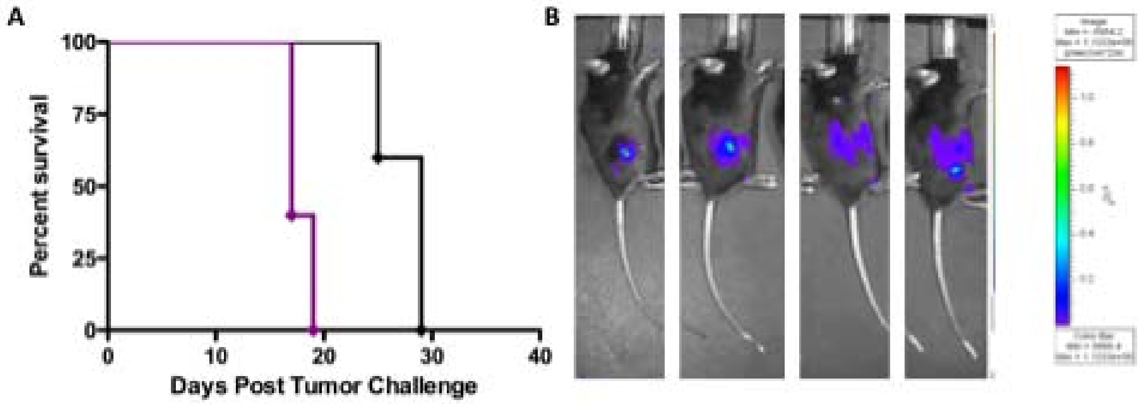

Figure 2: RM1-PSMA based mouse models of prostate cancer. (A) Disseminated tumor: 750 RM1-PSMA cells were injected i.v. into C57 BL/6 mice. One cohort was tumor-inoculated 1 week before 700cGys TBI (purple) and he second on the day of 700 cGy TBI (black). (B) Localized tumor: 5000 RM1-PSMA cells expressing luciferase were injected s. c. into C57BL/6 mice. The mice were injected i.p. with luciferin and bioluminescence monitored with a Xenogen IVIS system. Localized RM1 tumors could be visualized on Day 21 post tumor inoculation.

Figure 3

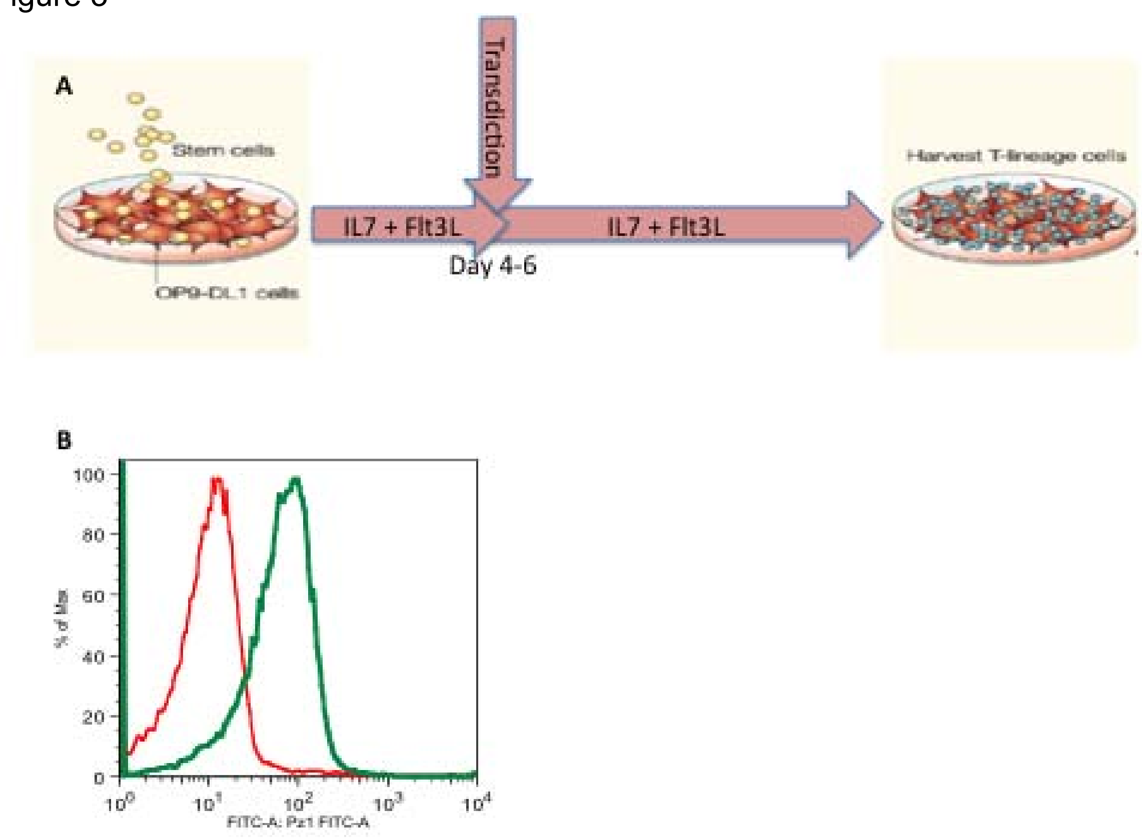

Figure 3: Genetic engineering of T cell precursors. (A) Scheme to transduce T cell precursors with Pz1 expression vectors (B) The co-cultured T cells were stained with idio-specific antibody directed to the analyzed 7 days post transduction.